extracted using guanidine thiocyanate-phenol-chloroform and treated with RNase-free DNase. RNA yield was determined spectrophotometrically.

mRNA expression analysis was performed as follows: 5 g of total cellular RNA were reverse transcribed by Moloney murine leukemia virus reverse transcriptase with random nucleotide hexamers. The RT mix includes reverse transcriptase, mRNA template, primers, buffer and dNTP's. Expression of the beta-actin housekeeping gene was used to determine cDNA yield and integrity and to check for possible contamination with genomic DNA. To this end, an intron-spanning beta-actin-specific primer set was designed: beta-actin forward primer SEQ ID NO: 4, 5'-GGC ATC GTG ATG GAC TCC G-3' and beta-actin reverse primer SEQ ID NO: 5, 5'-GCT GGA AGG TGG ACA GCG A-3' (amplicon length, 622 bp).

The resulting cDNA strand was directly used for amplification. A forward and a reverse primer were designed to hybridize specifically to the 5'- (forward) and 3'-region (reverse) of a cDNA encoding one of the aforementioned enzymes (C1GalT1 was amplified using forward primer: SEQ ID NO: 6, GAG ATT CCA GAG ATA CCA TTG and reverse primer: SEQ ID NO: 7,CGT TCA GGT AAG GTA GGT TG (amplicon length 262); C2GNT forward: SEQ ID NO: 8, GTG CTC AGA ATG GGG CAG GAT GTC ACC TGG, reverse: SEQ ID NO: 9, TCA CTA CTA GGA TTC TCC CCA GCA AGC TCC (amplicon length 360); ST3Gal-I forward: SEQ ID NO: 10, ATG AGG TGG ACT TGT ACG GC, reverse: SEQ ID NO: 11, AAC GGC TCC AGC AAG ATG (amplicon length 375); ST3Gal-II forward: SEQ ID NO: 12, CCC TGC TCT TCA CCT ACT CG, reverse SEQ ID NO: 13,: GCA TCA TCC ACC ACC TCT G (amplicon length 282); ST6Gal-I forward: SEQ ID NO: 14, AAA AAC CTT ATC CCT AGG CTG C, reverse: SEQ ID NO: 15,TGG TAG TTT TTG TGC CCA CA (amplicon length 379); ST6GalNAc-I forward: SEQ ID NO: 16, ACC ACA GCC AAG ACG CTC, reverse: SEQ ID NO: 17, AAG GGT GGT GCA AAG TGT TC (amplicon length 407); ST6GalNAc-II forward: SEQ ID NO: 18, CTG CCA GTA AA T TCA AGC TGC, reverse: SEQ ID NO: 19, TTG CTT GTG ATG AAT CCA TAG G (amplicon length 184)). The PCR reaction mixture contained 1.5 l of PCR buffer [100 mM Tris-HCl (pH 8.3),500 mM KCl], 0.8-1.0 l of 25 mM $MgCl_2$, 5 l of cDNA, 1.5 l of 2 mM deoxynucleotide triphosphates, 0.2 l of AmpliTaq polymerase, 0.2 l of 0.25 M beta-actin primer, 0.7-0.8 l of 50 M enzyme-specific primers and H_20 to a volume of 15 l. PCR run multiple temperature cycles for denaturation, annealing and elongation. Cycling conditions were 1 min at 94°C, 1 min at 59°C and 2 min at 72°C. beta-actin was co-amplified within the same tube with 0.2 l of beta-actin primer. Each PCR reaction was performed twice.

Aliquots of PCR reaction obtained after 26 to 28 cycles were electrophoresed in 2 % agarose gels and quantified via the intensity of ethidium bromide staining. For semiquantitative analysis of the RT-PCR data, the staining intensity of each cDNA-specific band was compared with the staining intensity of band corresponding to co-amplified beta-actin. The intensity of each enzyme band was correlated with the appropriate beta-actin band by calculating the ratio [(fluorescence units of the enzyme band/fluorescence units of beta-actin) x 100]. The detection limit was 1 ng of double-stranded DNA. The assay was linear up to 25 ng.